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Comparative analyses of ribosomal RNA genes of African and related trypanosomes, including *Trypanosoma evansi*

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Molecular phylogeny provides an alternative view on evolutionary relationships among related organisms. The comparison of the sequences of the ribosomal RNA genes (rDNAs) is a powerful tool for phylogenetic analysis. The rDNAs are one of the multicopy genes and are tandemly repeated in the genome (Fig. 1).

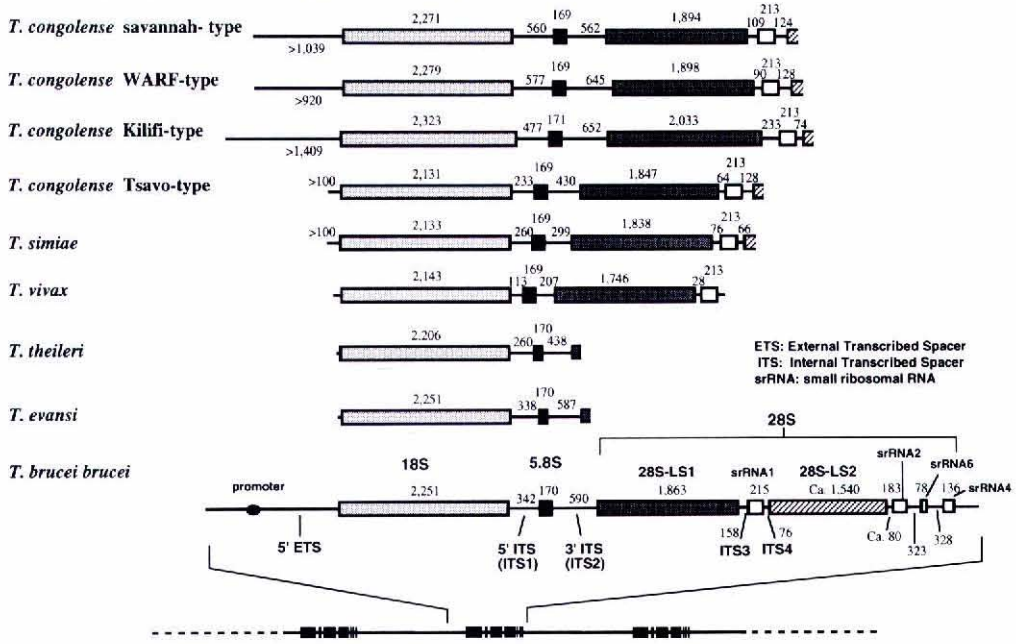

Figure. 1. The gene maps of different trypanosomes including *Trypanosoma evansi*. All sequence data, except for *T. brucei brucei*, were obtained in this study. The ribosomal RNA genes (rDNAs) are repeated in tandem and are transcribed as a long precursor RNA molecule starting from the transcription initiation site in the promoter sequence. Then the transcript is cleaved immediately into 18S, 5.8S, 28S-LS1, 28S-LS2, and small ribosomal RNAs by the specific RNases.

The unique characteristic of rDNAs is explained by the concerted evolution. According to the concerted evolution, genes are homogenized during evolution of species by homologous recombination, unequal crossover, sister chromatid exchange, gene conversion or other mechanisms. In order to investigate questions surrounding the phylogenetic relationships among African trypanosomes, we have analyzed rDNAs of 31 strains of 11 different trypanosomes: *Trypanosoma evansi* (8 strains), *T. b. brucei* (2), *T. b. gambiense* (7), *T. b. rhodesiense* (2), *T. congolense* [savannah-type (5), West African-riverine-forest type (1), Kilifi-type (1), Tsavo-type (1)], *T. vivax* (1), *T. simiae* (1) and *T. theileri* (2). The ribosomal RNA genes, including 18S rDNA, 5' ITS, 5.8S rDNA, 3' ITS, and 28S-LS1, were

obtained either as genomic DNA clones in lambda phage or as PCR products from trypanosome genomic DNA, and were either sequenced directly or sub-cloned into plasmid vectors prior to the sequencing. The phylogenetic trees were obtained by different analysis programs (Fig.2).

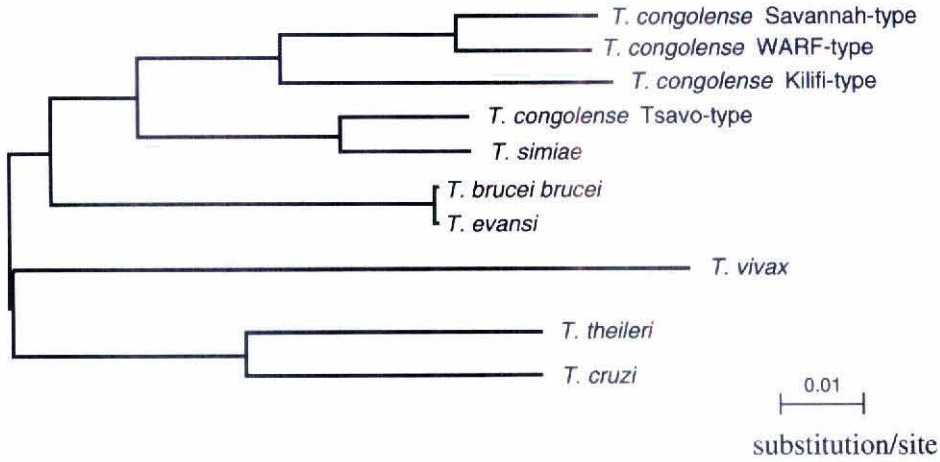

Figure 2. The phylogenetic tree of 18S rDNA. The tree was obtained by a small computer program, CLUSTAL W (ver. 1.74, Thompson JD, Higgins, DG, and Gibson TJ: Nucleic Acids Res, 22, 4673-4680, 1994). Four types of *T. congolense* were located as different branches. In contrast to this, the sequence differences were not observed between *T. evansi* and *T. brucei brucei* including *T. brucei gambiense* and *T. brucei rhodesiense*.

We first analyzed the rDNAs of four different types of *T. congolense*. There was significant heterogeneity among the sequences, making each of the trypanosomes clearly distinguishable on this basis. The sequence differences were observed in the spacer regions, the 18S and the 28S-LS1 rDNA segments. Among 8 isolates of savannah-type from the different parts of Africa, an insignificant number of nucleotide substitutions were observed in the 18S rDNA region. Of the different types of *T. congolense* (Accession Number: U22315), the West African-riverine-forest type (U22319) was found to be closest to the savannah-type, with 97% matches, followed by the Kilifi-type (U22317) with 93% matches.

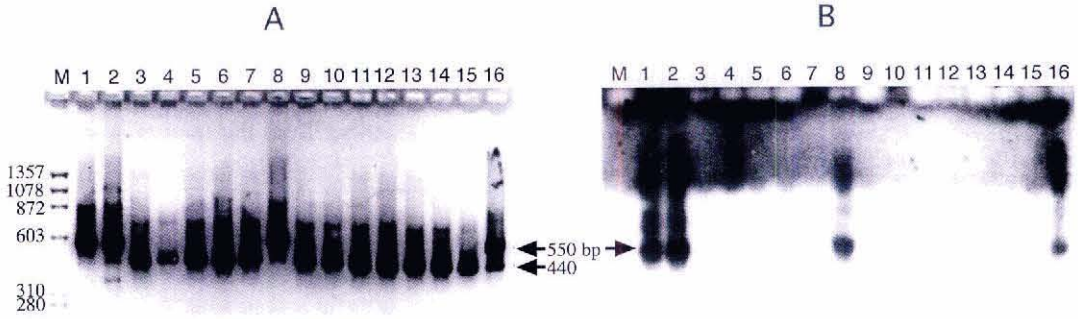

Figure 3. An application for diagnosis to distinguish species or types of trypanosomes using rDNA sequence data. The PCR amplification of the region of 5' ETS (external transcribed spacer) and Southern blot analysis were shown. Sixteen DNA samples were amplified by PCR using a pair of primers which amplifies 550bp in 5' ETS of *T. congolense* Savannah-type and 440bp of *T. congolense* Kilifi-type. A: 2% agarose gel electrophoresis of the PCR products. B: Southern blot probed with 700bp ETS DNA from the Savannah-type. The sample number 1, 2, and 8 were the Savannah-type. The sample number 16 were mixture of the Savannah-type and the Kilifi-type. The others were the Kilifi-type.

Curiously, the Tsavo-type (U22318) *T. congolense* was phylogenetically closer to *T. simiae* (U22320) than it was to the savannah-type. For instance, we could differentiate the Savannah-type and the Kilifi-type with ease using the differences of rDNA sequences (Fig.3). Our data strongly suggests that the four trypanosomes now grouped together as *T. congolense* could be classified into individual species.

This situation contrasts with what we observed in the analysis of *T. brucei* and related trypanosomes. The subgenus *Trypanozoon* comprises three species: *T. brucei*, *T. evansi* (U75507, D89527) and *T. equiperdum*. Within *T. brucei* three are subspecies: *T. b. brucei* (M12676), *T. b. gambiense* and *T. b. rhodesiense*. In addition to host range, the major phenotypic difference among trypanosomes in this subgenus is the absence of kDNA maxicircle, which is correlated with the capacity for cyclical development in the tsetse fly. We found a few nucleotide base substitutions in both the 18S rDNA and the spacer regions when the rDNA sequences of these trypanosomes were compared; we had no data for *T. equiperdum*. It is thought that *T. evansi* evolved from *T. brucei*, and then spread from Africa to other parts of the world in the recent past. Although the two trypanosomes have different lifestyles, we found no evidence from the analyses of rDNA genes to support their classification into different species even in the spacer sequences. For simplicity from evolutionary point of view they may be regarded as subspecies or variants of *T. brucei*.

In addition, it became clear that *T. vivax* (U22316) was located out of both the Salivarian and Stercorarian groups and *T. theileri* (AB007814) was close to *T. cruzi* (M31432) in the phylogenetic tree of 18S rDNA (Fig.2).

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